



The Cytochromes P450 and Mechanisms of Chemical Carcinogenesis

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The insidious and lethal nature of cancer, associated with its unique characteristics of dedifferentiation and loss of specialist function, invasion, and metastasis, is the result of changes in the chemical nature and structure of the proteoglycans of the cell glycocalyx and lysosomal enzymes, resulting from alterations in the highly specific glycosylations of the glycosyl transferases of the endoplasmic reticulum (1–3). These changes in cell proteoglycans, among the earliest changes seen in malignancy and used for histological diagnoses of premalignant states (4), are not only highly characteristic of the disease but also contribute progressively to the full establishment of malignancy. Changes occur in the glycocalyx, resulting in loss of cell adhesion (5) or in open intercellular tight junctions, enabling toxic chemicals and reactive intermediates to reach the critical basal cells of epithelia. These changes may evoke permanent DNA damage, resulting in a malignant cell clone. Changes in cell adhesion result in metastasis (3,6), and changes in the proteoglycan hydrolases are associated with tissue invasion (7,8). It is not insignificant that these earliest changes in glycosyl transferase activities and proteoglycan synthesis occur in the endoplasmic reticulum, the major site of metabolite activation of chemical carcinogens and of the generation of reactive oxygen species (ROS) from the futile cycling of the microsomal cytochromes. Furthermore, the endoplasmic reticulum is believed to contain its own DNA.

The categorization of cancer development into the phases of initiation, promotion, progression, and development is an oversimplistic approach, since cancer, like aging, is a process of continuous and progressive DNA degeneration (9,10) resulting from inherited impairment of DNA protection, repair, and regulation, DNA damage from ROS as in aging, and alkylation, arylation, and acylation of DNA by chemical carcinogens. These processes result in errors in transcription, leading to mutations and activation of proto-oncogenes (11), which are considered to be genotoxic mechanisms, and also lead to altered regulation of DNA transcription,

repair, and replication, often considered to be nongenotoxic. Consequently, cancer is probably the result of several different factors including inherited tendencies, aging, dietary deficiency in ROS scavengers and antioxidants, and exposure to toxic environmental chemicals. Putting aside inherited factors, which though most important cannot readily be changed, the major causative factors in cancer would appear to be ROS associated with the aging process, oxidative stress, degenerative disease, and chemical carcinogens.

Chemical carcinogenesis has long been associated with planar molecules, which may intercalate with DNA; with oxidative activation of the carcinogens by cytochromes P450 to reactive intermediates, which are electrophilic and bind covalently to DNA, thus damaging the genetic material and activating oncogenes; with the production of ROS, which may directly damage the DNA or activate carcinogens to reactive intermediates; and with activation of the protein kinase C cascade, leading to the phosphorylation of key nuclear proteins (transcription factors) involved in the regulation of DNA replication, changes in the epidermal growth factor, immunosuppression, dedifferentiation and hyperplasia (12,13). The activation of chemical carcinogens to reactive intermediates (ultimate carcinogens) is catalyzed primarily by cytochrome P4501 (CYP1) (14,15), which selectively accepts planar molecules (carcinogens) as substrates (16); oxygenates them in conformationally hindered positions, thus forming highly reactive epoxides, which, however, are not easily detoxified by epoxide hydrolase, glutathione transferase, and other detoxification enzymes (16); and is regulated by a cytosolic (steroidlike) receptor, the Ah receptor, which binds some carcinogens and other planar molecules to induce increased production of CYP1, Ah receptor protein, and other enzymes by genomic depression and also activates the protein kinase C cascade (14). Although the majority of known carcinogenic chemicals are believed to be metabolically activated by CYP1, the nitrosamines and other small molecules are activated by cytochrome P4502E1 (17).

This article reviews mechanisms of chemical carcinogenesis, from metabolic activation and generation of reactive oxygen species by cytochromes P4501 and P4502E to DNA damage, activation of protein kinase C and oncogenes, hyperplasia, and proteoglycan changes in the cell glycocalyx and lysosomal enzymes which mediate invasion and metastasis. *Key words:* chemical carcinogenesis, cytochrome P450, protein kinase C, reactive oxygen species. *Environ Health Perspect* 102:852–853 (1994)

Other mechanisms, including the action of prostaglandin synthetase (18), myeloperoxidase, and ROS-mediated oxygenations, are known to be equally effective in carcinogen activation. However, the unique pivotal role of CYP1 in chemical carcinogenesis is due to its coordination with the regulatory Ah receptor, resulting in the normal, low, non-injurious tissue levels of CYP1 being greatly augmented (1000-fold or more) by enzyme induction (19). Moreover, this enhanced metabolic activation of carcinogens by CYP1 to damage the DNA coincides with the activation of protein kinase C to augment DNA replication and evoke hyperplasia, thus ensuring that the faulty genetic information is preserved and perpetuated in a clone of DNA-damaged cells.

ROS can similarly damage DNA (20) and may also result in activation of protein kinase C, oncogene activation, and multi-stage carcinogenesis (21). ROS are generated continuously in biological systems by the low efficiency in the conversion of chemical energy into work, the associated electron leakage from membranes, and the consequent reduction of O₂ to superoxy anion and other ROS. As ROS generation depends on tissue O₂ uptake, and this is high in small animal species, the spontaneous formation of ROS is greatest in small rodents (22). Furthermore, biological systems have an antioxidant defense system to prevent ROS leading to oxidative stress, tissue damage, and mutations, and this is critically dependent on tissue glutathione concentrations. While small rodents, especially mice, consume tissue glutathione in chemical detoxification, humans and larger animals more expeditiously use water and epoxide hydrolase to detoxify carcinogenic epoxides, with the overall consequence that rats and mice are much more susceptible to the toxic effects

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of ROS, thereby exhibiting much higher levels of spontaneous cancer and much shorter life-spans than man and other large animals (23). Production of ROS may be greatly increased by futile cycling of the microsomal cytochromes P450; even phenobarbitone, which is regarded as a non-carcinogen and is metabolized by CYP2B, results in considerable ROS production, which can potentiate the malignancy of known carcinogens (e.g., dimethylnitrosamine).

The extreme case of P450 futile cycling is seen with cytochrome P450E1 (CYP2E1), an enzyme which is believed to result in the oxygenation of difficult-to-oxidize substrates (e.g., ethanol) by generating ROS in the vicinity of the substrate (24,25). Enhancement of CYP2E1 activity (by substrate-induced stabilization of the enzyme, not enzyme induction) leads to a prolonged burst of ROS production that can result in tissue necrosis, mutations, malignancy, organ failure, and death. Numerous chemicals are known to enhance CYP2E1 activity [e.g., ethanol, acetone, halothane, and many other small halogenated chemicals (26)] and hence to provoke ROS generation with consequent toxicity and carcinogenicity in small rodents. Indeed, inspection has revealed that those chemicals that cause cancer in rodents, especially mice, but not in larger species, are mostly substrates of CYP2E1. For the reasons given, these chemicals are unlikely to result in oxidative stress in humans and larger animals species, and are therefore unlikely to constitute as serious a carcinogenic hazard as substrates of CYP1.

By using molecular parameters (molecular planarity, collision diameters, frontier orbital energies) that discriminate substrates of CYP1 and CYP2E1 from each other and from those of other cytochromes, a novel procedure (COMPACT) has been developed to detect potential genotoxic carcinogens (CYP1 substrates) and rodent carcinogens (CYP2E1) (22,27,28). The lack of concordance between the Ames test for mutagens (which detects mostly CYP1 substrates) and the rodent two-species assay (which identifies both CYP1 and CYP2E1 substrates) is thus explained. Apart from this development in cancer detection and prevention, the understanding of the multifactorial mechanisms of chemical carcinogenesis has facilitated new preventive measures and treatments, including inhibition of CYP1 (29,30), enhanced detoxification of ROS (31,32), and dietary supplementation with antioxidant nutrients (31,33,34).

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